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CAP

(Compliance Audit Program)

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February 21, 1992

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Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460
Attention: Section 8(e) Coordinator (CAP Agreement)

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OTS DOCUMENT RECEIPT OFFICE

Subject: Occidental Chemical Corporation ("OxyChem")
Toxic Substances Control Act ("TSCA")
Section 8(e) Compliance Audit Program
Agreement No. 8ECAP-0063

Dear Sir:

Attached find one original and two copies of the following document:

- Dechlorane Plus Metabolism and Environmental Screening

This document is being submitted pursuant to the TSCA Section 8(e) Compliance Audit Program ("CAP") and a CAP agreement executed between OxyChem and the U. S. Environmental Protection Agency (Agreement No. 8ECAP-0063).

The identity of the chemical(s) tested in the study listed above are as follows:

- Dechlorane Plus, CAS# 13560-89-9.

The adverse effect(s) noted in the study listed above are as follows:

- A metabolism study in rats with 14C-labeled material demonstrated that Dechlorane Plus is retained in the tissue and is slowly excreted. Calculated Bioconcentration Factor based on the octanol-water partition coefficient was 7 million.

If you have any questions on the information contained herein, please contact me at (716) 286-3358.

Sincerely,

Ladd W. Smith

Ladd W. Smith
Director, Product Stewardship

Occidental Chemical Corporation
Corporate Environmental Affairs
Occidental Chemical Center
360 Rainbow Boulevard South, P.O. Box 728, Niagara Falls, NY 14302-0728
716/286-3000

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TITLE PAGE-STOCK, OTP

Dechlorane Plus

Metabolism and Environmental Screening Programs

SRI International 1978-79

Smead MFG. CO. HASTINGS MN - LOS ANGELES - CHICAGO LOGAN OH - MCGREGOR TX - LOCUST GROVE GA

FORM 7A

ROUTING
#1~~S. Gelfand~~
#2L. H. Schongar
#3G. A. Balsam
#4L. R. Walker

SCD MANAGEMENT REVIEW OF AGREEMENTS INVOLVING PROPRIETARY INFORMATION

The attached draft agreement plus one (1) copy has been prepared by the Patent/Legal Section at the request of Samuel Gelfand, for review and comment:

SECTION A
(To be completed by Originator)

1. Subject Dechlorane[R] Plus Mutagenicity Tests.

2. Reason for Agreement:

3. Reviewed and Authorized by: William J. Curtin Jr. 6/14/79
Division Patent Counsel Date

SECTION B

Please review the proposed agreement indicating all changes, corrections and/or comments which you feel should be made or considered. Comments for special consideration should be made in the space provided below:

1. Originator: Samuel Gelfand SG 4. June 8, 1979
Name Initiation Date

K L. H. Schongar

2. (Management level #1) _____
Date: _____ Initial: _____

3. G. A. Balsam
(Management level #2) _____
Date: _____ Initial: _____

4. L. R. Walker
V. P. General Manager _____

Approval:* Yes ___ No ___ Date: _____ Initial: _____

*If no changes or corrections have been noted, please execute and date both original and copy where indicated.

Please return this agreement to the Division Patent Counsel for appropriate handling of all changes and/or comments prior to ASAP.

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May 3, 1979

Dr. Daniel Scharf
Hooker Research Center
Long Road
Grand Island, New York 14072

Reference: SRI Project LSC-8060

Dear Dr. Scharf:

Two copies of our report entitled "Metabolism and Environmental Screening Studies on Dechlorane Plus" are enclosed. The proposal to perform the additional requested work is being reviewed by our contracts office and will be sent to you shortly.

Because the aqueous solubility values in the report differed widely, we performed another solubility experiment. The experiment was performed as described in the report, except that the sample centrifuging time was increased to 4 hours. Two samples were analyzed. For Isomer 1, we obtained solubility values of 44 ± 13 and 82 ± 12 ng/liter. For Isomer 2, we obtained values of 94 ± 46 and 277 ± 25 ng/liter. All glassware except the centrifuge tubes were thoroughly cleaned with hot methylene chloride and burned before use. The centrifuge tubes could not be treated this way without damage and we had no new ones. It appears that they were contaminated with D+ because the blanks for Isomer 1 and 2 contained 16 ± 6 and 55 ± 18 ng/liter of D+, respectively. These values were not subtracted from the solubility values reported above.

It is obvious that the aqueous solubility of D+ is difficult to determine with precision and accuracy.

Yours truly,

David Liu

David H. W. Liu, Ph.D., Manager
Aquatic Toxicology Program

Enclosures (2)

SRI International
333 Ravenswood Ave. • Menlo Park, California 94025 • (415) 326-6200 • Cable: STANRES, Menlo Park • TWX: 910-373-1246

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RECEIVED
OCT - 8 1979
HEALTH

28 September 1979

Dr. Paul Nees
Hooker Research Center
M.P.O. Box 8
Niagara Falls, New York 14302

Dear Dr. Nees:

I wish to clarify the statements made in the last paragraph of the summary section of our final report entitled, "Metabolism and Environmental Screening Studies on Dechlorane Plus."

In that paragraph, the statements concerning the potential hazard of Dechlorane Plus were based on the event that significant quantities of the compound are discharged to the aquatic environment. I understand that the compound was developed specifically for use as a fire retardant in plastic electrical insulation material, and that when incorporated into such material, it becomes tightly bound. Used electrical equipment and material are usually disposed of on land. It is thus unlikely that significant quantities of the compound will enter directly into the aquatic environment.

On land, the hazard of the compound should be very low. Because it binds tightly to plastic and has an extremely low aqueous solubility, it is unlikely that significant amounts will leach from discarded electrical insulation. Any quantity that does should sorb immediately and quite firmly to the soil, confining the compound to the disposal site.

Sincerely,

David H. W. Liu

David H. W. Liu, Ph.D., Manager
Aquatic Toxicology Program

cc: Daniel Scharf

SRI International
333 Ravenswood Ave. • Menlo Park, CA 94025 • (415) 326-6200 • Cable: SRI INTL MNP • TWX: 910-373-1246

SRI International

METABOLISM AND ENVIRONMENTAL SCREENING STUDIES ON DECHLORANE PLUS

Final Report

25 April 1979

By: Drs. T. W. Chou, D.H.W. Liu, W. R. Mabey, C. Mitoma, and J. H. Smith

Prepared for:

HOOKER RESEARCH CENTER
Long Road
Grand Island, New York 14072

Attention: Drs. Paul Nees and Daniel Scharf

SRI Project LSC-8060

Approved by:

David C. L. Jones, Director
Toxicology Laboratory

W. A. Skinner, Executive Director
Life Sciences Division

P. J. Jorgensen, Vice President
Physical and Life Sciences Division

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SUMMARY

The results of our metabolism and environmental screening tests on Dechlorane Plus (D+) are summarized below.

Aqueous Solubility

This parameter was difficult to determine. Direct results from solubility experiments with D+ indicated a solubility of 207 ng/liter for one isomer and 572 ng/liter for the other isomer. Indirect results from the sediment-water partitioning experiment suggested that the solubility was about 44 ng/liter (total for both isomers). This lower value was considered to be the best estimate of the aqueous solubility of D+.

Sediment Sorption

The compound has a very high affinity for particulate matter. Based on the sediment used in this study, its sediment-water partition coefficient is 4.5×10^6.

Photolysis and Oxidation in Water

Both reactions were very slow. The photolysis half-life of D+ was estimated to be >24 years; the oxidation half-life was estimated to be 2100 years.

Metabolism and Excretion by Rats

After oral administration of ^{14}C-labeled compound (or D+), measurable amounts of radioactivity were found in tissues and urine. The radioactivity was retained in the tissues and was slowly excreted. A metabolite was isolated from the liver extract but it was not identified.

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Accumulation in Fish

Steady-state (maximum) bioconcentration factor (BCF) values for D+, estimated from values for solubility and the sediment- and octanol-water partition coefficients, ranged from about 52,000 to 16,000,000. The BCF, estimated from a calculated octanol-water partition coefficient and using an EPA-recommended equation, was 7,000,000. Data from an exploratory bioconcentration test were inconclusive because of the presence of particulate D+ in the test solution.

Degradation By Sewage Sludge

The compound was degraded under aerobic conditions but not under anaerobic conditions. Considerable acclimation time (> 2 weeks) was required before the aerobic microorganisms began to metabolize the compound.

Interpretation of the Results

The data indicate that if D+ enters a natural body of water, it will probably persist for a long time. Most of it will concentrate in the sediment; up to 44 parts per trillion may be found in the water. Desorption from the sediment will tend to keep the concentration in the water relatively constant. Thus, D+ presents a long-term hazard to aquatic organisms as well as to man if drinking water supplies are contaminated with it.

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RECOMMENDATIONS

We recommend additional studies to further define the environmental fate and biological effects of D+. Suggested studies follow.

- Sediment Sorption. Sorption to sediment appears to be the major environmental fate of D+; however, this conclusion is based on experiments with only one kind of sediment. Additional sorption isotherms should be measured.

- Microbial Transformation. Metabolism of D+ by aerobic microorganisms appears to be the major route for removal of D+ from the aquatic environment. However, our biotransformation experiments were conducted under ideal conditions unlikely to be duplicated in the aquatic environment. The rate and extent of biotransformation of D+ in natural water of different types (oligotrophic and eutrophic) should be determined using frequent sampling to define acclimation and transformation patterns. Studies to identify and determine the acute toxicity of metabolites and studies to determine whether microorganisms can use D+ as a sole carbon source (as differentiated from cometabolism) should be considered.

- Metabolism and Excretion by Rats. The calculated Log P value for D+ indicates that it may be highly lipophilic and could therefore accumulate significantly in body tissues of animals on continuous or repeated exposure. The design of our exploratory experiment was not suitable to investigate this possibility. We recommend a repeated dose study as originally requested by Hooker Research Center. Our study showed that when D+ is administered orally to rats, almost 100% of the dose is eliminated through the feces. This suggests that D+ is not absorbed significantly from the gastrointestinal tract or that it is rapidly metabolized and eliminated

through the bile. The actual process can be readily identified by using bile duct cannulated rats and monitoring the radioactivity in the bile fluid.

- Carcinogenic Potential. D+ shows the potential for entering drinking water supplies and thus exposing humans to low levels of the compound for extended periods. The carcinogenic potential of D+ should be investigated using one or more screening tests for mutagenicity.

- Effects in Aquatic Organisms. The calculated bioconcentration factors (BCF) for D+ in fish suggest that the compound will concentrate in the tissues of fish to an equal or greater extent than compounds such as DDT or the PCBs. Our exploratory experiment was too short to determine the steady-stage BCF, and the static exposure technique appears unsuitable for D+. A full-scale bioconcentration test using the flow-through exposure technique is suggested. Long-term effects should also be studied using the Daphnia chronic test and/or the early life stage test with fathead minnows.

INTRODUCTION

At the request of Hooker Research Center, SRI International performed a study to obtain preliminary information on the probable impact of Dechlorane Plus (D+) on human health and the environment. The experiments were performed to determine the following:

- Aqueous solubility
- Sediment-water partitioning
- Photolysis in water
- Oxidation in water
- Uptake and distribution in rats
- Uptake by fish
- Degradation by sewage sludge.

The results of the study are reported herein. The experimental methods and data are presented in separate sections corresponding to each parameter.

We received three samples of D+: 8 g of unlabeled material (crystalized from benzene/carbon disulfide) bearing no lot number; 10 g of unlabeled material identified as Dechlorane Plus 25, Lot No. 7033C; and approximately 725 mg of ^{14}C-labeled material. The labeled material was received from Pathfinder Laboratories, Inc., St. Louis, Missouri, which assigned it a lot number of 80211; the other samples were received from the Hooker Research Center. According to information that accompanied the shipment, the radiolabeled sample contained 35 mCi of carbon-14 and had a specific activity of 31.5 mCi/mM.

Daniel J. Scharf, Manager, Functional Additives Research, Hooker Research Center, informed us in a letter dated 20 June 1978, that the 8-g sample of unlabeled material was not representative and should not be used. Hance, in the study we used only the radiolabeled material and the unlabeled material identified as Lot No. 7033C.

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METHODS AND RESULTS

Aqueous Solubility

We dissolved approximately 0.75 mg of D+ (Lot No. 7033C) in hexane and coated the walls of a clean 5-gallon glass carboy with the solution. The carboy was filled to the top with water from a Millipore water purification system and a teflon stopper with a glass syphon was put into the neck of the carboy. The contents of the carboy were allowed to equilibrate for about six weeks while being stirred slowly with a Teflon stirring bar and a magnetic stirrer. During this time, the temperature of the carboy was monitored and found to be 22° C ± 2.5° C.

The syphon was flushed with several hundred milliliters of water before a sample was taken. The sample, consisting of approximately 150 ml of D+-saturated water, was placed in clean centrifuge tubes and spun for one hour at 8,000 rpm (~5000 g). About two-thirds of the water in each tube was removed by a pipet placed 1/4" below the surface. The volume of water removed was measured in a graduated cylinder. Two centrifugings were necessary to get a 200-ml sample. The supernatant was extracted in a separatory funnel with 20 ml of 15% methylene chloride in hexane, which was also used to extract material from the walls of the pipet and graduated cylinder. The combined extracts were added to a Kaderna-Danish (K-D) concentration apparatus through a funnel filled with sodium sulfate to remove the water. The separatory funnel and the sodium sulfate were rinsed with several aliquots of the methylene chloride-hexane mixture and added to the K-D apparatus. The volume of the extract was reduced to 0.5 ml on a steam bath.

Analysis of the extracts was performed with a Hewlett-Packard Model 5830A gas chromatograph equipped with an electron capture detector, using the following conditions:

Column: 4' x 1/4" O.D. glass containing 5% OV-101 on 80/100 mesh Chromosorb G-high performance.

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Gas: 95% argon/5% methane - 50 ml/min
Oven temperature: 300° C
Injection temperature: 325° C
Detector temperature: 300° C.

The solvent flush technique was used to maximize the precision of the analyses.

D+ is a mixture of two isomers. The retention time was about 11 minutes for Isomer #1 and about 12.5 minutes for Isomer #2. Standards of D+ made in hexane were used to quantitate both isomers in the concentrated water extracts. The assumption was made that the response factors of the isomers are equal.

Two water extracts and a solvent blank were analyzed in triplicate. The results of the analyses are presented in Table 1. Note, however, that the solubilities presented in the table are probably overestimates because the solutions analyzed may have contained particulate D+ (see below).

Sediment-Water Partitioning

The D+ water solution used in this experiment was from the same source as that used in the water-solubility experiment. We centrifuged this solution at about 4500 g for 60 minutes. The supernatant was used for the sorption studies.

The sediment (from White Lake, Michigan) was provided by the Hooker Research Center. It was taken at Station 2-B at a depth of 35 feet on December 5, 1978; no additional information on it was provided. The sediment was a thick black ooze with a sulfurous odor and a high organic content. We sieved the sediment to remove large (>2 mm) debris, suspended it in water, and allowed the mixture to stand for 30 seconds. The suspended material was removed and the concentration of D+ in the sediment was measured. This suspension was then diluted to a concentration of 7.2×10^{-5} g/ml for use in the sorption isotherm.

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Table 1

CONCENTRATION OF ISOMERS #1 AND #2
IN WATER SATURATED IN D+

	Isomer #1 (ng/liter)	Isomer #2 (ng/liter)
Sample #1	211	623
	210	603
	236	685
Mean	219	637
95% Confidence range	± 37	±106
Sample #2	193	507
	198	515
	192	502
Mean	194	508
95% Confidence range	± 8	± 16
Pooled Data		
Mean	207	572
95% Confidence range	± 13.6	± 63.3
Blank	Insignificant	Insignificant

Three mixtures were prepared. One mixture was a blank containing D+ and water, but no sediment. The other two were duplicates and contained D+, water, and sediment. The mixtures were made as follows:

	Volumes Used (ml)		
Mixture	D+ Centrifuged Solution	Water	Sediment Suspension (7.2×10^{-5} g/ml)
1	200	200	0
2	200	196	4
3	200	196	4

The mixtures were shaken overnight and then centrifuged at about 4500 g for 90 minutes. The supernatant (top 300 ml) was extracted with 15% CH_2Cl_2 in hexane. In Mixtures 2 and 3, the bottom 100 ml and the centrifuge tubes were extracted together. The bottom 100 ml of Mixture 1 was extracted separately from the centrifuge tubes. The solvent extracts were filtered through anhydrous Na_2SO_4 and concentrated using a K-D apparatus. The concentrated solvent extracts were analyzed by the same procedures as those used in the determination of the water-solubility of D+.

Table 2 summarizes the analytical results. The abnormally high amount of D+ in Sample 1-BL compared to Sample 1-TL indicates that particulate D+ was present. Since Sample 1 was diluted by half with water and centrifuged about 2.5 times longer than the samples in the solubility experiment, the samples in the solubility experiment probably also contained particulate D+. The value for Sample 1-TL probably represents a better estimate of the solubility of D+ in water than the values for the two isomers presented in Table 1. Thus, we estimate the solubility of D+ in water to be $(4.41 \pm 0.02) \times 10^{-5}$ µg/ml or 44.1 ± 2 ng/liter.

The calculation of the amount of D+ sorbed on the sediment in Samples 2 and 3 was corrected for the amount of soluble and particulate D+ in the bottom of the centrifuge tube:

Table 2

ANALYTICAL RESULTS OF ISOTHERM

Sample Number*	D+ in Solvent Extract (µg)	Volume Extracted (ml)	C_w (µg/ml)	C_s (µg/g)	K_p (C_s/C_w)
1-TL	13.2×10^{-3}	300	4.40×10^{-5}		
	13.4×10^{-3}		4.43×10^{-5}		
1-[illegible]	37.5×10^{-3}	100			
	42.1×10^{-3}				
1-CT	1.57×10^{-3}	1 Tube			
2-TL	6.56×10^{-3}	300	2.19×10^{-5}		
	8.96×10^{-3}		2.99×10^{-5}		
2-BL+S+CT	43.3×10^{-3}	100		74	3.5×10^{6}
	45.6×10^{-3}			106	
3-TL	7.09×10^{-3}	300	2.36×10^{-5}		
	7.57×10^{-3}		2.53×10^{-5}		
3-BL+S+CT	47.4×10^{-3}	100		133	5.5×10^{6}
	47.6×10^{-3}			136	

* TL = Top liquid
BL = Bottom liquid
S = Sediment
CT = Centrifuge tube.

$$D+_{sorbed} = D+_{BL+S+CT} - D+_{particulate} - D+_{soluble}$$

$$D+_{soluble} = D+_{TL}/3$$

$$D+_{particulate} = D+_{1-BL} - D+_{1-TL}/3 .$$

All the D+ was assumed to be in either the sediment, the particulate, or in solution. A check of the amount of D+ adsorbed to the walls of the centrifuge tubes showed only relatively small amounts in the blank containing no sediment. It was assumed that D+ would preferentially absorb to the sediment in those samples where sediment had been added. We calculated the sorption partition coefficient (K_p) and estimated error to be $(4.5 \pm 1.9) \times 10^6$.

Photolysis in Water

We determined the rate of photolysis of D+ in hexane and in water. First, we performed a preliminary experiment in which a hexane solution of D+ was irradiated for two weeks and its gas-liquid phase chromatograph (glpc) trace was compared with that of an identical solution kept in the dark (control). This is a procedure we have recently adopted for compounds that we expect to have low aqueous solubility and low photolability. It permits use of a higher, and thus a more easily quantitated, concentration of compound and provides information on whether work with aqueous solutions is then necessary.

Second, using information obtained from the preliminary experiment as a guide, we irradiated two 1-ppm solutions of D+ for 168 hours. One solution was prepared with distilled water, and the other was prepared with natural water from a nearby eutrophic lake. We added acetonitrile (5% of total volume) to both solutions to completely solubilize D+. The chromatograms of the two solutions were compared with those of identical solutions kept in the dark (control).

To photoirradiate the solutions, we used a 450-watt mercury lamp with a borosilicate immersion well. The borosilicate well also served

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as a filter allowing only wavelengths above 290 nm to reach the solutions. This irradiation system does not duplicate the photon flux and wavelength distributions of natural sunlight; however, it does provide several lines of high photon fluxes in the solar spectral region. With this system, phototransformation occurs more rapidly than in natural sunlight and thus facilitates the experiment.

Analyses for D+ and its phototransformation products were performed with a glpc equipped with an electron capture detector. Before analysis, the aqueous solutions were extracted with a methylene chloride-hexane mixture, and the extract was reduced to 1.0 ml using a K-D evaporator.

The photoirradiation system was roughly calibrated using p-cresol as a reference compound. In a previous study (Smith et al., 1978), we determined the half-life for p-cresol to be 70 days when exposed to natural sunlight (spring season) with a quantum yield of 0.08. The rate constant for photolysis of p-cresol in the above system was 7.5×10^{-2} hr^{-1}.

In the preliminary experiment with D+ in hexane, the chromatograms of the irradiated and control solutions of D+ were similar except that in the irradiated solution, we found an early eluting peak that represented 15% of the total integrated area in the chromatogram of the irradiated solution. This peak was detectable but not integratable in the control chromatogram. By analogy to photolysis of other bicyclic chlorinated hydrocarbons, we believe that this peak may represent a reduced product, which can result from photodissociation of a C-Cl bond followed by H-atom abstraction from the hexane solvent. The total areas under the two D+ peaks in the chromatogram of the control solution and the total areas of the two D+ peaks plus that of the greater peak in the irradiated solution each comprised about 95% of the total integrated area in their respective chromatograms. This suggests that the greater peak represents a phototransformation product of D+.

In the experiments with aqueous solutions of D+, the chromatograms of the irradiated and control solutions were nearly identical: the

integrated areas under the two D+ isomer peaks amounted to 63 to 67% and 21 to 24% of the total integrated area in each chromatogram. Although we did not use an internal standard during the analyses, we estimate that no more than 10% could have been lost to photolysis. If an error occurred, we believe it to be from the uncertainty in the volume (1.0 ± 0.1 ml) to which the extracts were reduced in the K-D evaporator.

The chromatograms of the photolyzed and control aqueous solutions showed the same early-eluting peak found in the photolyzed D+/hexane solution, but this peak was not as great as that found in the latter. The greater peak in the hexane solutions may be attributable to some pecularity in the hexane solvent experiment (such as induced photoreduction) or to the possibility that in water, photodissociation of the C-Cl bond of D+ leads to products that are not completely extractable with methylene chloride-hexane. In any event, a 16% increase in the concentration of the apparent photoproduct (15/95 x 100%) in the irradiated D+/hexane solution after 2 weeks is within our extimated 10% error for the 1-week experiment with the aqueous solutions.

An upper limit on the photolysis half-life of D+ in aquatic systems can be estimated from the above information using the following reasoning and assumptions:

1. If we assume that the experimental error in our analyses was 10%, then at least 90% of the D+ remained after 168 hours of photolysis in our photolysis apparatus. Assuming the photolysis of D+ follows first-order kinetic behavior, as is usually encountered for chemicals in dilute solution, the photolysis rate constant for D+ in our system is $< 6 \times 10^{-4}$ hr^{-1}, which corresponds to a half-life of >48 days.

2. The equation for calculating the photolysis rate of a chemical is

$$k_p = 2.3\phi\Sigma\varepsilon_\lambda I_\lambda \ .$$

where ϕ is the reaction quantum yield that measures the efficiency of the photolytic process in converting absorbed light (i.e., energy) into chemical reaction, and the term $\Sigma\varepsilon_\lambda I_\lambda$ is the sum of the products of the light intensity I_λ and the absorption coefficients of the chemical ε_λ in specific wavelength intervals centered at the wavelength λ. To estimate the sunlight photolysis rate of D+, we assume that for the photolysis of D+ and p-cresol in the same light source, the $\Sigma\varepsilon_\lambda I_\lambda$ terms are equal; this, then, assumes that the difference in photolysis rates of D+ and p-cresol is due to the quantum yields.

3. The maximum possible reaction quantum yield for D+ photolysis can then be estimated by reference to p-cresol data (designated by c)

$$\phi_{D+} = \frac{k_p^{D+}\phi_c}{k_p^c} = \frac{(<6 \times 10^{-4})(0.08)}{(7.5 \times 10^{-2})} = <6 \times 10^{-4}$$

and the photolysis rate constant from (1) above. It should be remembered that this quantum yield is based on a limit of detectability of the D+ photolysis reaction, and the quantum yield may be much smaller than 6×10^{-4} as calculated above.

4. The limiting half-life for photolysis of D+ in aquatic systems may also be estimated using the equations and assumption stated in (2) above.

$$t_{\frac{1}{2}} = \frac{\ell n 2}{2.3\phi\Sigma\varepsilon_\lambda I_\lambda}$$

$$t_{\frac{1}{2}}^c = \frac{\ell n 2}{2.3\phi_c\Sigma\varepsilon_\lambda I_\lambda}, \quad t_{\frac{1}{2}}^{D+} = \frac{\ell n 2}{2.3\phi_{D+}\Sigma\varepsilon_\lambda I_\lambda}$$

$$t_{\frac{1}{2}}^{D+} = \frac{(t_{\frac{1}{2}}^c)\phi_c}{\phi_{D+}} = \frac{(70)(0.08)}{(<6 \times 10^{-4})} = >24 \text{ years}$$

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It is thus apparent that D+ photolysis in aquatic systems is very slow.

Oxidation in Water

Before we actually perform experiments to determine the rate of oxidation of a compound in water, we customarily estimate the first-order rate constant and the half-life of the compound to determine whether the experiments are necessary. We estimated the first-order rate constant of D+ to be (10^{-8} x 10^{-3}), or 10^{-11} sec^{-1}, which is equivalent to an oxidation half-life of 2100 years.

A recent study showed that the concentration of peroxyl radicals in sunlit natural water ranges from 10^{-8} to 10^{-9} M, and that the first-order rate constants for the oxidation of cycloalkanes like D+ are in the order of 10^{-3} to 10^{-4} M^{-1} sec^{-1} (Hendry et al., 1974). In estimating the rate constant for D+, we used the higher values for both the peroxyl radical concentration (10^{-8} M) and the cycloalkane rate constant (10^{-3} M^{-1} sec^{-1}). We decided that it was unnecessary to experimentally measure the half-life of D+ because it was clear that peroxyl radical oxidation of the compound is not an important transformation process.

Metabolism and Residue Studies

We prepared a suspension of ^{14}C-D+ by mixing approximately 10 mg of the compound with 5 ml of water containing 5% each of Tween-80 and gum arabic. Analysis of this suspension showed that it contained 27.6 µCi (0.57 mg) of ^{14}C-D+ per 0.5 ml.

We administered 0.5 ml of the suspension orally to each of six Sprague-Dawley rats that weighed between 160 and 200 g each. Three of the rats were kept together in a large metabolism cage and sacrificed 4 hours posttreatment. The remaining rats were kept in individual metabolism cages and sacrificed 24 hours posttreatment.

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The levels of radioactivity in samples of the blood, urine, and a homogenate of the liver were determined directly after solubilizing the samples in Scintisol (Isolab, Inc.). Before radioanalyzing samples of dried feces and a homogenate of the kidney, we combusted them in a Packard Tri-Carb Oxidizer (Model 8306). All samples were analyzed in duplicate. Radioanalysis was performed with a Searle Analytic Mark III liquid scintillation counter. The level of radioactivity in the whole organs, body fluid, and feces were determined by multiplying the radioactivity found per unit weight or volume of the radioanalyzed subsample by the weight of the organs and feces or by the total volume of the collected fluids. The total volume of blood was estimated.

To determine whether the radioactivity found in the liver was D+ or a metabolite, we pooled the liver homogenates from the rats sacrificed 4 hours posttreatment and extracted the pooled sample with 3 volumes of ethyl acetate. The extract was dried over sodium sulfate and then further dried with a stream of nitrogen. The dark brown syrupy residue was dissolved in benzene-ethanol (1:1) and chromatographed on a Sephadex G-15 column (1 x 20 cm). The column was eluted in succession with about 10 ml each of benzene, benzene-ethanol (1:1), and ethanol. The colored, 5-ml portions of eluate in the first four tubes were discarded. The colorless eluate in the fifth tube contained a sufficient amount of radioactivity to concentrate and analyze by thin-layer chromatography (tlc). We spotted a silica gel 60F plate with a ^{14}C-D+ standard, the liver extract, and a mixture of the standard and liver extract and developed it using cyclohexane-acetone (9:1) as a solvent. The plate was then autoradiographed for several days.

Table 3 presents the levels of radioactivity (expressed as the percentage of the administered amount) in the blood, kidneys, liver, urine, and feces of the six orally treated rats. The data show that very little of the administered compound is absorbed from the gastrointestinal tract. An average of 94.6 percent of the administered radioactivity appeared in the feces. However, when absorbed, D+ is excreted very slowly as evidenced by virtually no change in the level

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Table 3

DISTRIBUTION OF TOTAL RADIOACTIVITY

(Percent of Administered Amount)

Time Post-Treatment	Blood*	Kidney	Liver	Urine	Feces
4 Hours	1.6×10^{-2}	2.0×10^{-3}	3.3×10^{-2}	2.8×10^{-4}†	
	1.5×10^{-2}	0.7×10^{-3}	3.8×10^{-2}	8.3×10^{-4}†	
	1.2×10^{-2}	0.4×10^{-3}	2.1×10^{-2}	6.3×10^{-4}†	
24 Hours	0.7×10^{-2}	0.7×10^{-3}	1.5×10^{-2}	6.0×10^{-3}	76.13
	1.4×10^{-2}	1.5×10^{-3}	4.6×10^{-2}	5.7×10^{-3}	104.8
	1.0×10^{-2}	1.5×10^{-3}	3.1×10^{-2}	3.5×10^{-3}	103.01

[redacted]

* Calculated on the basis of 8% of the body weight representing blood.

† Urine in bladder.

radioactivity in the blood, kidneys, and liver between 4 and 24 hours post-treatment.

Table 4 shows the concentration of radioactivity, expressed as disintegrations/min/gram (dpm/g), in the blood, liver, and kidney. The higher amount in the liver than in the blood or kidney suggests that this organ may be the major site of metabolism and elimination of D+. Because the molecular weight of the compound is about 700, some of the compound and its metabolites would be expected to be eliminated through the bile. Hence, we believe that part of the radioactivity in the feces represents metabolites excreted via the bile.

Table 4

TISSUE CONCENTRATION OF RADIOACTIVITY
(dpm*/g)

Time Post-Treatment	Blood	Liver	Kidney
4 Hours	667	2796	799
	598	3287	268
	460	1841	173
24 Hours	345	1348	312
	540	3366	496
	391	2309	526

* Disintegrations/minute.

That D+ is metabolized by the rat was evident from the autoradiograms of the tlc plate after the liver extract had been chromatographed. The autoradiogram showed that the D+ standard had an R_f of 0.48, with minor contaminants at R_f of 0.23 and 0.04. The liver extract had an R_f of 0.33, which was clearly different from the R_f values for the contaminants, therefore indicating that the compound was a metabolite of D+. R_f is defined as the ratio of the distance traveled by the zone (spot representing the chromatographed material) and the distance traveled by the solvent front.

Bioaccumulation in Fish

We exposed 12 bluegill sunfish (Lepomis machrochirus) for 96 hours to 30 liters of dechlorinated tap water containing an average of 283 dpm/ml of ^{14}C-D+. We radioanalyzed two water samples and three whole fish each at 48 and 96 hours. The other six fish were transferred to a 19-liter aquarium and maintained in clean, flowing water for the next 96 hours. During this clearance period, three fish each were radioanalyzed at 48 and 96 hours. No water samples were collected.

Exposure occurred in a covered cylindrical glass container, and the test solution was aerated gently throughout the exposure period. The fish were not fed during the 192-hour experiment.

All the samples were frozen and the radioactivity was determined at the end of the experiment, using a Searle Analytic Mark III liquid scintillation counter after thawing the samples. The water samples were analyzed directly in Scintisol (Isolab, Inc.). The fish were weighed and prepared for radioanalysis by homogenizing them in 5 volumes of distilled water and combusting a measured volume of the homogenate in an oxidizer (Packard Tri-Carb, Model B306).

The results, presented in Table 5, were inconclusive primarily because of extreme variability in the measured levels of radioactivity in replicate water and fish samples and the absence of a logical pattern in uptake and depuration.

We believe that the problem was due to the presence of particulate D+ in the test solution. To achieve a measurable level of radioactivity in the test solution, we had to use an excess amount of ^{14}C-D+. Our attempt to prepare a true solution with measurable levels of radioactivity failed. In that trial, we dissolved the labeled material in hexane, coated the bottom of the exposure chamber with the hexane solution, and evaporated off the hexane. We added 30 liters of dechlorinated tap water to the chamber, stirred it continuously for several days, and radioanalyzed a sample of water daily. None of the samples contained a detectable level of radioactivity. To achieve

Table 5

LEVEL OF RADIOACTIVITY IN FISH
EXPOSED TO ^{14}C-D+

Phase and Sampling Time	Average Radioactivity (dpm)/ Gram of Sample		
	Fish	Water	BCF*
Uptake			
48-Hour	1,798 ± 1,281	256 ± 224	7.02
96-Hour	614 ± 47	311 ± 219	1.97
Depuration			
48-Hour	25,843 ± 33,314	--	--
96-Hour	1,299 ± 1,235	--	--

* Radioactivity in fish divided by radioactivity in water.

a detectable level, we added more of the hexane solution directly to the water. This produced a suspension of D+, as evidenced by the presence of fine white particles.

We believe that different fish ingested different amounts of the particles, giving rise to the large variation in the level of radioactivity among the fish. Nonhomogeneous distribution of the particles also caused problems in achieving reproducible counts for the water samples.

Several equations have been developed to calculate the steady-state (maximum) BCF of organic compounds from known values for the aqueous solubility, sediment-water partition coefficient, and octanol-water partition coefficient. These equations are used primarily to obtain a rough estimate of the BCF.

An equation proposed by EPA (Federal Register, 1979) is

$$\text{Log BCF} = 0.76 \text{ Log P} - 0.23 \qquad (1)$$

where P is the octanol-water partition coefficient. Kenaga and Goring (1978) determined the relationship between experimentally derived BCF

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values and experimentally derived values for aqueous solubility, the soil-water partition coefficient, and the octanol-water partition coefficient, and developed the following equations to express the relationships:

$$\text{Log BCF} = 2.791 - 0.564 \text{ Log WS} \quad (2)$$

where WS equals the aqueous solubility of the compound in mg/liter.

$$\text{Log BCF} = -1.579 + 1.119 \text{ Log } K_{oc} \quad (3)$$

where K_{oc} is the sediment-water partition coefficient.

$$\text{Log BCF} = -1.495 + 0.935 \text{ Log P} \quad (4)$$

To calculate the BCFs, we used the following equation input values:

- Water solubility: 4.4×10^{-5} mg/liter (revised value) and 3.89×10^{-4} mg/liter (average of the original values obtained for the two isomers of D+).
- Sediment-water partition coefficient: 4.4×10^{6}.
- Octanol-water partition coefficient: 1.99×10^{9}.

All of the values, except the octanol-water partition coefficient (P), were derived experimentally in this study. We calculated P using a computerized program developed at SRI for chemical structure-activity research. The calculated BCFs are presented in Table 6. Although there is poor agreement in the BCF values, all indicate that D+ could concentrate significantly in fish, and to a greater degree than some well-known compounds such as DDT, chlordane, dieldrin, hexachlorobenzene, Aroclor 1016 and 1242, which have respective BCFs of 61,600 (Hansen and Wilson, 1970), 11,400 (Parish et al., 1976), 5,800 (Reinert, 1972), 8,600 (Macek et al., 1976), and 49,000 (Hansen et al., 1975).

Degradation by Sewage Sludge

We determined the magnitude of degradation of D+ by aerobic and anaerobic sewage sludge microorganisms. Both kinds of organisms were obtained from a sewage treatment plant in Menlo Park, California.

0 0 2 9

Table 6

STEADY-STATE BCF VALUES CALCULATED FROM VALUES FOR AQUEOUS SOLUBILITY, SEDIMENT-WATER PARTITION COEFFICIENT, AND OCTANOL-WATER PARTITION COEFFICIENT FOR D+

Equation Used	Equation Input		Calculated BCF
	Value	Parameter	
2	4.4×10^{-5} mg/l	WS	174,985
2	3.89×10^{-4} mg/l	WS	51,761
1	1.99×10^{9}	P	6,886,523
4	1.99×10^{9}	P	15,867,189
3	4.4×10^{6}	K_{oc}	716,182

The effluent containing aerobic organisms had a pH of 7.1 and a total suspended matter (TSM) concentration of 185 mg/liter. The pH and TSM for the effluent with anaerobic organisms were 6.9 and 3910 mg/liter, respectively.

In the aerobic degradation experiment, we placed 100 ml of aerobic effluent in each of several 250-ml Erlenmeyer flasks and injected D+ into each flask with a Hamilton microsyringe. One series of flasks received 10 µl of benzene containing 21.8 µg of D+ with 0.89 µCi of carbon-14; another series received 40 µl of benzene containing 87.2 µg of D+ with 3.55 µCi of carbon-14. The final concentration of D+ in the flasks in the two series was 218 and 872 µg/ml, respectively. For controls, we used flasks that had been sterilized in an autoclave after introducing the activated sludge. The D+ concentrations are well above the limit of the aqueous solubility of D+; hence, most of the compound was in suspension.

The flasks were sealed with cotton plugs and incubated at 25° C in a temperature-controlled shaker. Some of the flasks were incubated for 2 weeks and the rest for 6 weeks. After incubation, duplicate flasks were removed from the shaker and the contents were extracted and analyzed for radioactivity and by thin-layer chromatography (tlc).

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In the anaerobic degradation experiment, we used 125-ml Erlenmeyer flasks with 100 ml of anaerobic effluent in each. The amount of ^{14}C-D+/flask was the same as in the aerobic degradation experiment. The flasks were flushed with nitrogen gas and sealed with rubber stoppers, each of which was equipped with a glass outlet tube connected to a test tube containing 10 ml of 0.5N KOH to trap radioactive CO_2. The flasks were incubated statically at 35° C (the normal temperature in the anaerobic digester at the Menlo Park Sewage Plant) for 2 and 6 weeks.

We extracted the contents of the 2-week incubated flasks three times with 60 ml of hexane-isopropanol (3:1) and washed the pooled extracted material with 50 ml of water to remove isopropanol. The aqueous fraction was adjusted to pH 2 with 10% phosphoric acid and extracted twice with 60 ml of ethylacetate. We found the hexanol-isopropanol extraction procedure to be somewhat inefficient and tedious, so for the 6-week incubated flasks we used benzene (40 ml, 3 times) instead of hexanol-isopropanol as the primary extractant.

We measured the volume of the two solvent fractions and the aqueous fraction and removed a 1-ml aliquot from each for radioanalysis (liquid scintillation). The remaining volume of each fraction was dried over sodium sulfate, evaporated under vacuum at 40° C, and concentrated to 2 ml with a stream of nitrogen gas at room temperature. Aliquots (50 to 100 µl) of the concentrated fractions were spotted on silica gel tlc plates and developed with cyclohexaneacetone (9:1). The developed plates were autoradiographed.

Tables 7 and 8 present the radioanalytical results of the aerobic degradation experiment. Both the aerobic and anaerobic experiments were designed to determine whether degradation occurred by examining the relative distribution of radioactivity in each of the fractions. We used hexane-isopropanol or benzene to extract the nonpolar components (primarily D+) and ethylacetate to extract the polar compounds. The presence of radioactivity in the ethylacetate extract and the aqueous fraction would indicate the presence of metabolites.

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Table 7

PERCENT RADIOACTIVITY OF ADDED ^{14}C IN EXTRACTION FRACTIONS OF 2-WEEKS-INCUBATED AEROBIC BIODEGRADATION TEST FLASKS

Flasks	Viable		Sterile	
Added ^{14}C level (x 10^6 dpm)	1.97	7.88	1.97	7.88
Hexane-isopropanol fraction	50.5%	58.5%	72.3%	81.6%
Ethylacetate fraction	21.3%	19.0%	4.0%	3.7%
Aqueous fraction	5.3%	8.1%	0.2%	0.2%
TOTAL	77.1%	85.6%	76.5%	85.5%

Table 8

PERCENT RADIOACTIVITY OF ADDED ^{14}C IN EXTRACTION FRACTIONS OF 6-WEEKS-INCUBATED AEROBIC BIODEGRADATION TEST FLASKS

Flasks	Viable		Sterile
Added ^{14}C level (x 10^6 dpm)	1.97	7.88	7.88
Benzene fraction	32.2%	0.10%	85.7%
Ethylacetate fraction	10.1%	0.05%	6.0%
Aqueous fraction	0.6%	0.01%	0.4%
TOTAL	42.9%	0.16%	92.1%

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The presence of radioactivity in the ethylacetate and aqueous fractions (Table 7) suggested that D+ had been metabolized; however, no labeled metabolites were present on the tlc plates. The only labeled compounds on the plates were D+ and its contaminants. Examination of the autoradiograms of the tlc plates of all fractions from both experiments (aerobic and anaerobic, 2- and 6-week) also failed to reveal the presence of metabolites.

We therefore decided to use the difference between the total radioactivity recovery values in the viable and sterile (control) flasks as an indication of biodegradation. This decision was based on the hypothesis that if metabolism occurs, D+ is rapidly converted to CO_2 and water, for which we had some evidence (see below).

Using this method of evaluating the data, we concluded that little or no degradation of D+ occurred in the 2-week aerobic flasks (Table 7), but that a high percentage was degraded by the aerobic microorganisms in the 6-week flasks (Table 8). Under anaerobic conditions, no degradation occurred (Tables 9 and 10); also, no radioactivity was found in the CO_2 traps.

Using some of the 6-weeks-incubated aerobic flasks, we attempted to determine whether D+ was indeed metabolized to CO_2 and water by installing a CO_2 trap in several flasks. The average amount of radioactivity recovered from the trap was about 1% of the total added radioactivity. Installation of the trap prevented adequate aeration; hence, D+ was metabolized very slowly. Nevertheless, it appears that CO_2 is a metabolic product of D+.

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Table 9

PERCENT RADIOACTIVITY OF ADDED ^{14}C IN EXTRACTION FRACTIONS OF 2-WEEKS-INCUBATED ANAEROBIC BIODEGRADATION TEST FLASKS

Flasks	Viable		Sterile	
Added ^{14}C level (x 10^6 dpm)	1.97	7.88	1.97	7.88
Hexane-isopropanol fraction	77.1%	85.4%	66.2%	62.8%
Ethylacetate fraction	11.9%	5.7%	12.4%	16.2%
Aqueous fraction	0.4%	0.5%	6.3%	6.5%
TOTAL	89.4%	91.6%	84.9%	85.5%

Table 10

PERCENT RADIOACTIVITY OF ADDED ^{14}C IN EXTRACTION FRACTIONS OF 2-WEEKS-INCUBATED ANAEROBIC BIODEGRADATION TEST FLASKS

Flasks	Viable		Sterile
Added ^{14}C level (x 10^6 dpm)	1.97	7.88	7.88
Benzene fraction	75.3%	71.6%	75.1%
Ethylacetate fraction	12.1%	11.2%	13.0%
Aqueous fraction	0.1%	1.3%	0.3%
TOTAL	87.5%	84.1%	88.4%

DISCUSSION

Information provided to us by the Hooker Research Center on the acute and subacute toxicity of D+ to laboratory mammals and on the acute toxicity of the compound to aquatic organisms and some of the information obtained from this study suggest that D+ presents a relatively low acute hazard to human health and to aquatic organisms. We believe, however, that the compound should be evaluated for possible chronic effects on human health and in aquatic organisms if there is any possibility that D+ will be a contaminant of the aquatic environment.

Although the aqueous solubility of D+ is very low (>1 ppb), the compound shows the potential for being highly persistent in the aquatic environment. Thus, if the compound enters a natural body of water, it will probably remain there for a long time. Most of the compound will probably sorb to the bottom as well as suspended sediments; however, as the concentration of the compound in the water column declines through dilution and other processes, desorption from the sediment will tend to keep the concentration in the water column relatively constant.

If the water from the aquatic system is used for drinking, humans could be exposed chronically to low levels and perhaps adversely affected. Our study on the metabolism and excretion of D+ in rats showed that only a small percentage of D+ is absorbed from the gastrointestinal tract after a single dose. However, metabolism and excretion of the absorbed fraction was very slow. The estimated high Log P value (9.3) for D+ suggests that repeated exposure will cause the compound to accumulate in the tissues. The extent could be about 2000 times that of DDT (Log P $\sim$ 6). The propensity of D+ to bioconcentrate also poses a long-term hazard to aquatic organisms.

REFERENCES CITED

Federal Register. 1979. Water Quality Criteria, Request for Comments, 44(52), 15973 (15 March 1979).

Hansen, D. J., S. C. Schimmel, and J. Forester. 1975. Trans. Amer. Fish. Soc. 104, 584-588.

Hansen, D. J., and A. J. Wilson. 1970. Pesticides Monitoring Journal 4, 51-56.

Hendry, D. G., T. Mill, L. Piszkiewicz, J. A. Howard, and H. K. Eigenmann. 1974. J. Phys. and Ch[illegible]. Ref. Data 3, 937-978.

Kenaga, E. E., and C.A.I. Goring. 1978. Relationship between water solubility, soil-sorption, octanol-water partitioning, and bioconcentration of chemicals in biota. Presented at the Third Aquatic Toxicology Symposium, ASTM, New Orleans.

Macek, K. J., M. A. Lindberg, S. Sauter, K. S. Buxton, and P. A. Costa. 1976. USEAP-600/3-76-009, 57 pp.

Parrish, P. R., S. C. Schimmel, D. J. Hansen, J. M. Patrick, Jr., and J. Forester. 1976. J. Toxicol. Env. Health 1, 485-494.

Reinert, R. E. 1972. J. Fish. Res. Bd. Can. 29, 1413-1418.

Smith, J. H., W. R. Mabey, N. Bohonos, B. R. Holt, S. S. Lee, T.-W. Chou, D. C. Bomberger, and T. Mill. 1978. Environmental Pathways of Selected Chemicals in Freshwater Systems. Part II. Laboratory Studies. EPA Report EPA-600/7-78-074.

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5-9-79

#8,900 - SRI for Mutagenicity

Project Personnel

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Hydrolysis D William Maybey 4670

Rat metabolism Dr. Mitoma 2232

Sewage Sludge Dr. Chou (Wen) 2950

fish uptake Dr. D. Liu

D. Plus Hazard - Secondary after

low solubility! - could cause problem in transport to body cell

Ames no - test

∴ not able to get effectively into cell

hooker

INTER-OFFICE MEMORANDUM

To: W. J. Crossetta

Copies to:

Subject:

File Ref.:

Date: April 14, 1978

From: D. J. Scharf

Div./Dep't: Corp. R&D

Location: Grand Island

Enclosed find a proposal and agreement forms prepared by SRI that relate to an Environmental Contract for work entitled, "Metabolism and Environmental Screening Studies on ^{14}C-Labeled Dechlorane Plus ". The proposal and costs have the recommendation of both Paul Nees, (Environmental Dept.) and Arun Bhattacharya, (R&D Environmental liason for Dechlorane Plus).

These documents are sent to you for your review. Take particular note that the revised contract calls for a $20,000 cost vs $56,750 in the original proposal.

Please prepare the appropriate secrecy agreement forms and send them to me. I will obtain the proper R&D approvals and then forward your document to SRI.

Thanks for your cooperation.

Dan Scharf

Daniel J. Scharf
lcl

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SRI Proposal LSC 78-26 (R2)
21 March 1978

Dr. Arun K. Bhattacharya
Research and Development
HOOKER RESEARCH CENTER
Long Road
Grand Island, New York 14072

Dear Dr. Bhattacharya:

On 16 March, Dr. Paul Nees, your corporate toxicologist, visited SRI International to discuss SRI Proposal LSC 78-26(R) with Dr. James Smith, Dr. David Bomberger, and me. The major emphasis of the discussion was on reducing the charge for the proposed work on Dechlorane Plus (D+) to no more than $20,000. Our original cost estimate (quoted in SRI Proposal LSC 78-26) was $56,750; on request, we modified some of the experimental protocols, deleted others, and submitted a revised cost estimate of $22,000 in SRI Proposal LSC 78-26(R). Based on decisions made during the 16 March meeting, our cost estimate is now $20,000.

The new cost estimate reflects a $2,000 reduction in the charge for conducting studies on the movement of D+ in soil and water. These studies entail determination of the aqueous solubility of D+ and its sediment-water partition coefficient. We will determine aqueous solubility as described in the original proposal because this information is essential to most of the other proposed studies. However, we will determine the sediment-water partition coefficient on a best-efforts basis; we will endeavor to obtain data that will provide an estimate of the magnitude of sorption of D+ to sediment.

Other proposed work includes the determination of D+ oxidation and photolysis, a screening study on absorption of D+ by rats and fish, and a microbial degradation screening study using activated sewage sludge. The methods

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we propose to use are described in SRI Proposals LSC 78-26 and LSC 78-26(R).

The contractual provisions presented in the original and revised proposal, copies of which are attached, apply to this second revision. This revision will remain in effect until 30 April 1978; however, SRI International would be pleased to consider an extension if requested.

Respectfully submitted,

David H. W. Liu

David H. W. Liu, Ph.D., Manager
Aquatic Toxicology Program

Approved:

W A Skinner

W. A. Skinner, Executive Director
Life Sciences Division

Attachments: SRI Proposals LSC 78-26 and LSC 78-26(R)

METABOLISM AND ENVIRONMENTAL SCREENING STUDIES ON ^{14}C-LABELED DECHLORANE PLUS (D+)

A Proposal for Research: LSC 78-26

10 February 1978

By: Drs. David C. Bomberger, Tsong-Wen Chou
David H. W. Liu, William R. Mabey,
Chozo Mitoma, and James H. Smith

Project Leader:

David H. W. Liu, Ph.D., Manager
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Paul J. Jorgensen, Vice President
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CONTENTS

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INTRODUCTION

SRI International is pleased to submit this proposal in response to the request from Hooker Research Center (dated 9 January 1978) to conduct selected studies on Dechlorane Plus (D+). We will address five subject areas pertaining to the environmental fate and effects of D+: movement in soil and water, degradation in the environment, mammalian metabolism and residues, environmental bioaccumulation and magnification, and plant uptake.

Most of the experiments will involve the use of ^{14}C-labeled D+, which we understand HRC will provide in addition to unlabeled D+. In reviewing the information on D+ provided in the RFP, we noted that the product has several impurities. The presence of these impurities, particularly if they become radiolabeled during the synthesis of D+, will create problems in our interpreting the data from the experiments. We request that HRC provide us with labeled and unlabeled D+ of high purity and with information on the amount and kinds of impurities present in the final product and whether or not they are labeled. We would be pleased to purify the product and will submit a cost estimate upon request.

Because of the expected low aqueous solubility of D+, the radiolabeled product must be of reasonably high specific activity to allow us to perform the experiments properly. If we assume that D+ is soluble in water to the extent of 5 ppb, then a specific activity of 20 mCi/mmole of D+ will give a solution containing 240 dpm and 4 ng of D+ per milliliter. This is the minimum level of activity required to conduct meaningful studies. If D+ is less soluble than 5 ppb, the specific activity would have to be adjusted accordingly. If our requirements cannot be met, use of a gas chromatographic technique would be required for quantitation of D+ in all aspects of the project. In that case, our cost estimate would have to be revised.

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TECHNICAL DISCUSSION

Movement in Soil and Water

Determination of Aqueous Solubility

The solubility of a compound in water determines its maximum exposure concentration for aquatic toxicity tests and for chemical and biological transformation rate studies. (We presume that uptake or transformation of solid materials is slow compared with the corresponding rates of dissolved material.) If the solubility of a solid material is low, the rate of its uptake or transofmration may depend partly on the rate of its dissolution. The rates of chemical and biological transformations as well as equilibrium processes such as sorption and volatilization cannot be measured correctly if solid material is present. Therefore, one of the first tasks in this project will be to measure the solubility of D+.

We have measured the solubility of mirex in water and obtained a value of $(70 \pm 10) \times 10^{-12}$ g ml^{-1} (ppt) at about 22° C. The solutions were prepared by the method of Haque and Schmedding (1975 Bull. Env. Contam. Toxicol. 14:13). This entailed dissolving the solute in an organic solvent and coating it on the side walls of a large carboy by allowing the solvent to evaporate while the carboy was rotated. The carboy was filled with water, and the solution was allowed to stabilize for several days. The equilibrated aqueous solution was centrifuged at 10,000 rpm for 1 hour and immediately extracted. The centrifugation step was required to obtain reproducible results; presumably, at these extremely low concentrations, particulate solute was suspended in the water. The aqueous solution was not stable because solute sorption to the walls was very rapid. The extract was concentrated and analyzed by electron-capture gas chromatography.

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May et al. (1978 Analyt. Chem 50:175) suggest an alternative procedure for preparing solutions of low-solubility compounds. The solute is coated on glass beads that are placed in a stainless-steel column. Water is pumped through the column and saturated with the solute. The method was successful for anthracene and phenanthrene. However, the solubility of these compounds is many orders of magnitude higher than what we expect for D+. We experienced difficulties with sorption of mirex to metal and glass, and the problems with D+ will probably be greater. Therefore, this method may not be satisfactory for producing saturated solutions of D+.

We will attempt to use the method of May et al., both for determining solubility and for preparing aqueous solutions of D+ for use in the sediment/water partition studies and the soil percolation studies. We hope the technique will prove satisfactory because it is less expensive to perform than the technique we used for mirex. If it does not prove satisfactory, the latter technique will be used, however.

Sediment/Water Partitioning

Adsorption phenomena are characterized as both equilibrium phenomena and kinetic phenomena. Equilibrium is frequently modeled using a Freunlich isotherm

$$C_s = K C_\ell^{1/n} \qquad (1)$$

where C_s is the concentration of solute on the soil (µg/g) and C_ℓ is the solute concentration in the liquid phase (µg/cm^3). Frequently the exponent turns out to be nearly 1, and when the liquid phase is water its density is 1 g cm^{-3}, so that Eq. (1) becomes

$$C_s = K C_\ell \qquad (2)$$

and K is a dimensionless partition coefficient. For strongly adsorbed materials such as mirex, K can be on the order of 500,000, and for

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weakly adsorbed materials it is less than 1. Some solutes show different partition coefficients when K is being measured by adsorbing solute from the liquid onto the soil compared with when it is being measured by desorbing material from the soil into the liquid.

Adsorption (or desorption) equilibrium is not obtained instantaneously. Times to equilibrium may vary from minutes to hours, depending primarily on the size (diffusion coefficient) of the solute and the microscopic structure of the individual soil particles. Equilibrium requires that the solute diffuse to all the adsorption sites that may be inside a particle and connected to the outside by microscopic pores, the diameters of which can vary from 1000 Å to 1 Å.

We have observed that the magnitude of K for natural sediments increases as the solubility decreases, and for nonpolar solutes it increases with the organic content of the sediment. Because we expect the aqueous solubility of D+ to be less than that of mirex (70 ppt), the partition coefficient of D+ should be greater than that of mirex (460,000) using the same kind of sediment. We will determine whether this prediction is true.

Because the RFP emphasizes that our efforts be directed to screening rather than to performing a comprehensive investigation, we will determine the sorption partition coefficient using only one kind of sediment. We recommend that HRC consider a more comprehensive study to be performed later with at least two sediments containing different amounts of organic carbon.

To determine the sediment sorption partition coefficient for D+, we will use sediment from Coyote Creek, a stream close to Menlo Park. We have used sediment from this creek in many studies of this type, including the sediment sorption studies with mirex, and have characterized it.

We will prepare aqueous solutions of D+ and equilibrate it with the sediment. A preliminary isotherm experiment will be run to determine the order of magnitude of K, the partition coefficient. This

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procedure will be followed by a definitive test using several flasks, as follows:

	Number of Flasks		
D+ Concentration	No Sediment	Low Sediment	High Sediment
None	1	2	2
Low	2	2	2
High	2	2	2

The suspensions in the flasks will be allowed to equilibrate for 12 hours. Then they will be centrifuged at 10,000 rpm for 1 hours, decanted, and extracted. We will measure the sediment and supernatant concentrations independently for each flask. The amount of sediment will be calculated on a dry-weight basis. The sorption partition coefficient will be calculated using a linear least squares regression method that fits Eq. (2).

Soil Percolation Study

Movement of a solute through a soil column is a function of the solute-soil interaction and the dispersion that results both from diffusion and mixing during flow through the tortuous pores in the soil. When chemical reactions do not occur, the major solute-soil interaction is adsorption, which can take place either on the soil surface and by interaction with soil organic material or through ion-exchange reactions with sites on the organic or inorganic functions of the soil.

The most common time when the movement of a material through soil needs to be measured is when the material has been placed on the soil surface, either through spillage or intentional application as an agricultural chemical. Ideally, the rate at which the material would be carried into and through the soil is calculated using adsorption partition coefficients, adsorption kinetic constants, and soil properties such as porosity and dispersion coefficient. Agreement between predictions and measurements are best for materials with large partition

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coefficients, because then the effects of dispersion and adsorption kinetics--which are hard to model correctly--are minimized.

In screening the potential for a material to migrate through the soil, the minimum experimental determinations involve measurement of K. If K is large, predicting the movement of the material should be possible using the mathematics developed to describe chromatography and ion-exchange phenomena. The minimum confirmation of material movement is a simple percolation test in a soil column. If the measured and predicted rates of transport disagree, then a series of experiments involving additional isotherms and studies of adsorption kinetics as well as a series of soil percolation tests are required to gain an understanding of the transport process and to determine whether the percolation test gave the correct result. A particularly important aspect of the additional testing is to verify that the soil columns were uniformly packed and did not have channels that allowed bypassing and rapid movement of the test material through the column. We will conduct two kinds of tests--the isotherm screening experiment and the simple soil percolation experiment.

Isotherm Screening Experiment

Our experimental protocol for the soil adsorption isotherm is basically the same as that described for sediment adsorption. A stock solution of D+ will be prepared and placed in several flasks along with measured amounts of soil. After an overnight contact period, the soil and supernatant will be separated and analyzed for D+. The sediment and supernatant concentrations will be fitted to the Freundlich expression.

A soil sample will be air dried and passed through a 2-mm sieve. Only the < 2-mm fraction will be used for isotherm measurements because the adsorption activity is concentrated in the fine fraction of the soil and the larger particles do not permit measuring uniform samples for the isotherm determination. A separate sieving experiment will be conducted to determine the weight percentage of the two sizes

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of fractions so that the adsorption partition coefficient can be corrected to a whole soil value.

A stock solution containing a known amount of ^{14}C-labeled D+ at a concentration below its solubility limit will be prepared. The ^{14}C activity of this stock solution will be confirmed by scintillation counting.

The isotherm experiments will be conducted by preparing flasks that contain suitable amounts of stock solution, distilled water for dilution, and a weighed amount of soil. All experiments will be run in duplicate, and flasks containing no soil and no stock solution will be included as controls. A preliminary isotherm experiment will be run using a low ratio of soil to D+ to determine the order of magnitude of the partition coefficient; then a series of flasks will be prepared to yield 50 and 25% adsorption from each of two D+ concentrations in solution. This will produce four duplicated points to determine the coefficients of the isotherm expression.

Analysis will include direct determination of the material in the supernatant solution (C_ℓ) and direct determination of the adsorbed material (C_s) to eliminate bias due both to volatilization of material during the equilibration period and its adsorption onto glassware. C_ℓ will be determined by centrifuging to remove the soil, decanting the supernatant, and then washing out the soil pill. An organic solvent extract of the supernatant and the centrifuge tube are used to determine C_ℓ, and an organic solvent extract of the soil and the water used to wash it from the centrifuge tube are used to determine C_s. All analyses will be by scintillation counting, and mass balance will be calculated to indicate whether losses occurred during the experimental procedures.

After the isotherm is completed, a mathematical prediction of soil transport will be calculated using

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$$\frac{\delta C_\ell}{\delta t} = \frac{D}{W}\frac{\delta^2 C_\ell}{\delta Z^2} + \frac{1}{W}\frac{D}{\Theta}\frac{\delta A}{\delta Z} - \frac{q}{\Theta}\frac{dC}{dZ} \qquad (3)$$

$$W = 1 + \frac{\rho}{\Theta N} K_A C_\ell^{(1/N - 1)} \qquad (4)$$

$$C_s = K_A C_\ell^{1/N} \qquad (5)$$

which has an exact analytical solution.

In this equation:

Θ = Water in soil (cm^3/cm^3)

Z = Depth in soil profile (CW)

D = Soil dispersion coefficient (cm^2/sec)

K_A = Adsorption partition coefficient.

Soil Column Percolation Experiment

Based on the molecular weight and similarity of D+ to mirex, we expect the adsorption partition coefficient to be large and the transport rates in soil to be low. Under these conditions, the soil percolation test has to be designed to detect migration when no material migrates through the entire soil column, meaning that no material would be detected in the column effluent. The soil column is set up so that, after an appropriate amount of solution has been passed through it, plugs containing soil and the interstitial water can be removed from various depths in the column for analysis. For compounds that migrate rapidly through the soil, this procedure provides a concentration profile both in the solid and liquid phase that can be interpreted in terms of the actual adsorption kinetics, irreversibility, and soil dispersion. For strongly adsorbed materials the procedure provides only a coarse indication of how far the material migrated.

A glass column approximately 4.5 cm in diameter and 30 cm long will be prepared. It will be sealed with a rubber stopper at the

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top and sealed at the bottom with a removable glass frit. At 2-cm intervals, access ports will be provided to permit removal of a plug of soil at the end of the percolation test. These ports will be sealed with teflon stoppers during the test.

Air-dried and sieved (< 2 mm) soil will be packed into the column using a vibratory apparatus to ensure uniform density. Free space will be left at the top for a liquid layer during percolation.

The column will be saturated with a weak (~0.01 N) calcium sulfate solution by forcing water through the column under pressure until the measured inflow rate at the top equals the effluent rate at the bottom. A measured amount of ^{14}C-labeled D+ will be introduced in a single spike, displaced into the column, and allowed to equilibrate overnight. Then pumping will be resumed at a rate equivalent to percolation of a year's rainfall* through the soil column in 2 days. Column effluent will be collected in fractions in case the material does break through, counter to expectations. These fractions will be analyzed only if necessary. At the end of the percolation period, the flow will be stopped and plugs of soil and interstitial solution will be removed from the column for analysis. The same procedures as those used for the isotherm will be followed. If the D+ does not appear in any of the samples removed from the column, the effluent fractions will be analyzed. If D+ is not in any of the effluent fractions, we will remove the soil from the column in layers, starting at the top (because the D+ will be between the points where plugs were removed), and analyze it.

If the D+ moves a substantial distance through the soil column, we will conduct a series of experiments involving more isotherms, kinetic studies, and soil percolation tests to determine more precisely how D+ interacts with the soil. These tests, if conducted, will require additional funding.

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* To be determined after discussion with HRC.

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Degradation in the Environment

Chemical Transformation Studies

Examination of the structure of D+ suggests that photolysis and free radical oxidation may occur in aquatic environments; hence, our chemical transformation experiments will be limited to the study of these two processes. Several isomers of D+ may be present in the commercial-grade product, but their reactivity toward oxidation or photolysis should be sufficiently similar that no difficulties will arise in screening tests; isomers could be a problem in detailed studies, especially if product analysis is required.

Hydrolysis of D+ in aquatic environments is unlikely because the bridgehead and vinylic chlorides in the molecule can only be hydrolyzed under extreme conditions; hydrolysis studies therefore will not be conducted.

Two matters relating to our proposed experimental work require comment. We consider the use of ^{14}C-labeled D+ unnecessary in the chemical transformation screening tests. We anticipate that gas chromatography using flame ionization or electron-capture detector will give a simpler and less ambiguous analysis of D+ in the reaction and work-up conditions.

Since the solubility of D+ in pure water is certainly in the low part-per-billion concentration range, we may use 1% acetonitrile as a cosolvent to increase the solubility of D+ in the screening studies. This solvent was used successfully in transformation studies of mirex and is inert under the conditions of our photolysis and oxidation experiments. Concentrations of D+ in all experimental work will be 1 ppm or less.

Photolysis

We recommend that two photolysis screening tests be performed with D+. One test should be carried out in pure water and the other in water that simulates a natural water. We propose this approach because

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natural materials in aquatic systems can promote photochemical reactions through photosensitized or photoinitiated free radical reactions. The photolysis in pure water will be carried out at wavelengths greater than 280 nm to determine whether direct photolysis occurs. The direct photolysis experiment will also serve as a control for the photolysis in the simulated natural water.

To screen the susceptibility of D+ to direct and indirect photolysis, experiments will be carried out in a merry-go-round photochemical reactor; the light source will be a 450-watt Hg lamp with a borosilicate glass immersion well that allows passage only of wavelengths greater than 280 nm. D+ at concentrations of 1 ppm or less will be photolyzed in a solution of water and in a solution of water containing 8 ppm humic acid. The solutions will be analyzed as required to follow the disappearance of the starting concentration of D+. The photolyses will be carried out for a maximum of about 1 week, which is approximately equivalent to 4 to 6 months in sunlight. At least four but not more than eight data points will be taken for each reaction solution. Aliquots of these two solutions will be kept in the dark during the photolyses as controls to demonstrate that no processes other than photolysis are responsible for any reactions found.

These experiments will determine whether direct or indirect photolyses of D+ occurs. Based on analogy with other photochemical studies at SRI using chemicals that had tailing absorbances in the region above 280 nm, we will be able to estimate whether the direct photolysis half-life of D+ in sunlight is 6 months or greater. Through these experiments, we will also determine whether indirect photolysis of D+ is important compared with direct photolysis. If either direct or indirect photolyses are important, we will then recommend appropriate detailed studies that will more precisely describe the photolysis of D+ in the aquatic environment.

Oxidation

D+ may be oxidized by hydrogen transfer from the cyclooctane ring or by oxy radical addition to the chlorine-substituted double bond.

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Although the hydrogen transfer by peroxy radical is slow (half-life of several years), other aspects of oxidation of D+ are unknown and require an oxidation screening test. The proposed oxidation test is one developed at SRI during our oxidation research; we believe it is the best test currently available for quantitative measurement of the susceptibility of a chemical to free radical oxidation in aquatic environments.

We will conduct a free radical oxidation screening test on D+. This test is carried out in water under conditions that allow a quantitative estimate of the susceptibility of D+ to free radical oxidation in aquatic environments.

To screen the susceptibility of D+ to free radical oxidation, we will oxidize the chemical using azobis(methyl isobutyrate) (MAS) as the source of free radicals. Solutions of D+ at concentrations below its solubility limit will be prepared in pure water that contains 1.00×10^{-4} M MAB. The solutions, saturated with air, are placed in a 50° C water bath for 100 hours. Solutions of D+ at the same concentration without MAB are run simultaneously as controls under identical reactions conditions. The 100-hour reaction time corresponds to about two half-lives for MAB. The solutions are then analyzed for starting chemical.

The rate of oxidation of D+ is proportional to the concentration of $RO_2\cdot$ radicals generated by thermal decomposition of MAB. Kinetic analysis of reaction indicates that the rate of oxidation of D+ at 25° C will be nearly one-ninth as fast as at 50° C and the half-life nine times as long as at 50° C. Furthermore, the rate at 25° C can be related to a probable rate of oxidation in the aquatic environment.

Biodegradation

We will study the potential for biological transformation using activated sludge obtained from a local wastewater treatment plant that receives a mixture of municipal and industrial waste. Biological activity will be detected by changes in the extraction efficiency of

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the radioactivity in a series of organic solvents with a range of polarities. We have chosen this procedure rather than chromatography because we expect that little or no conversion to protein will occur and that the alcohol and epoxide products will have low solubilities.

Samples of activated sludge will be placed in a 20-liter bench scale activated sludge unit and maintained on an artificial sewage of diluted Carnation Instant Breakfast. (Carnation Instant Breakfast is often used in studies of this type because it has a balance of fat, protein, and carbohydrate and is easy to handle.) The unit will be operated for several days before sampling to develop a uniform and actively growing culture, so that if repeated experiments are required the sludge used will have the same properties. The laboratory growing period will also purge inorganic solids from the sludge. For a biological transformation experiment, sludge will be withdrawn from the unit, centrifuged, washed, centrifuged, and resuspended as a dilute suspension (several hundred parts per million suspended solids) that will be oxygenated.

A series of flasks will be prepared in duplicate containing aliquots of the aerated cell suspension, aerated dilution water, and aliquots of the ^{14}C-labeled D+ stock solution. One of each pair of flasks will be sealed with pure oxygen in the gas space and allowed to incubate for 24 hours on a shaker apparatus. This time period is not long enough to provide adaptation of the culture to growth on D+ as the sole carbon source, but it is long enough to show any biological transformations that would occur because of enzymes present in a resting cell culture. The other flask of each pair will be analyzed immediately.

For analysis, an aliquot of the flask contents will be extracted after sonication to rupture the cell walls. The sonicated mixture will be extracted with toluene, ethyl acetate, and butanol. The residual will be digested with an organic base to solubilize the organic material and eliminate suspended material. All extracts and residuals will be analyzed by liquid scintillation counting. If biological transformation occurs, it will be indicated by a change in

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the distribution of the radioactivity. For example, if biological activity converts some of the D+ to an alcohol, more of the total radioactivity will be present in the butanol extract of the incubated samples than in the butanol extract of the unincubated samples. The procedure will detect biological activity even if extraction efficiencies are not 100%.

The actual size of samples incubated will depend on the specific activity of the ^{14}C-labeled D+, and the appropriate sludge levels will have to be determined by some screening experiment. If biological activity is detected, at least a pair of flasks will be prepared and tested again to confirm the experimental results.

If significant biological transformation occurs, we will extend the approach to obtain an estimate of the biodegradation rate constant. To do this, we will withdraw aliquots from the incubation flasks at predetermined intervals and radioanalyze them. The radioanalytical data will be analyzed as if a second-order decay process were occurring. Thus, if S is the concentration of untransformed D+ and X is the sludge concentration,

$$\frac{dS}{dt} = k_b XS \qquad (6)$$

where k_b is the transformation rate constant. A simple second-order process is assumed because the D+ levels present will not be adequate to provide significant bacterial growth.

The proposed study does not include anaerobic transformation experiments since we believe that because of its physical properties D+ will probably always remain in the aerobic zone of the environment. If, however, D+ is released into a wastewater stream that enters a sewage treatment plant equipped with an anaerobic digestor, D+ could be exposed to anaerobic conditions. We would be pleased to determine the action of sewage sludge on D+ under anaerobic conditions for an additional charge. The method is the same as described above, except that D+ is incubated in an oxygen-free environment.

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Mammalian Metabolism and Residue Studies

The objective of this study is to define absorption, tissue accumulation, excretion, and possible metabolism of orally administered D+ in the rat. We will use young adult male Sprague-Dawley rats purchased from Simonsen Laboratories, Inc., Gilroy, California. Before experimentation, the animals will be acclimatized to metabolic cages for a few days.

The animal study will be conducted sequentially, starting with the absorption study. A preliminary experiment will be conducted using several oral doses of ^{14}C-labeled D+ to ascertain absorption of the compound by monitoring the blood radioactivity level. If confirmatory data are obtained that the compound is absorbed through the gut, a detailed pharmacokinetic study as proposed below will be conducted. If evidence for absorption is not obtained, the proposed protocols on distribution and elimination and chronic study will not be used. The metabolism study may be carried out after consultation with the client, if the metabolic effects of intestinal flora on the compound are of interest.

Absorption

Preliminary studies will be conducted to ascertain the rate and extent of absorption of D+ in the rat. Rats will be administered a suspension of ^{14}C-labeled D+ (5 to 10 μCi) by gavage, and blood samples will be taken from the orbital sinus 1, 2, 3, 4, 6, 8, and 24 hours after administration. The blood samples will be counted in a liquid scintillation counter. The quench-corrected counts per minute or the microgram equivalent of the administered D+ will be plotted on a semilog graph paper as a function of time. From the plotted data, the time of maximum concentration, the maximum concentration attained in the blood, and the half-life of ^{14}C-labeled D+ in the blood can be estimated. These data will be used to choose the time to sacrifice the rats for conducting tissue distribution studies.

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Tissue Distribution and Elimination

Twelve rats will be administered 5 to 10 uCi of ^{14}C-labeled D+ by gavage, and they will be kept in individual metabolism cages for separate collection of urine and feces. Groups of four rats will be sacrificed when the blood level is at a peak and when the blood levels are 50 to 60% and 10 to 20% of the peak level. These times will be chosen on the basis of the results obtained from the absorption study. The blood and major organs (kidney, liver, heart, lung, spleen, muscle, testes, brain, fat, skin), the gastrointestinal tract, and the excreta will be analyzed for radioactivity contents. The data will be expressed as the percentages of the administered dose found in each whole organ or tissue and radioactivity per gram of each tissue. The blood-to-tissue ratios and the biological half-life of D+ in each organ will be determined graphically.

Chronic Exposure

To assess the effect of repeated intake of D+ on the pharmacokinetic behavior of the compound, rats will be fed a diet containing unlabeled D+ (0.1 to 0.5% in the powdered Purina Chow diet) for 2 weeks. The rats will then be given a single oral dose of ^{14}C-labeled D+. Control rats maintained on powdered Purina Chow diet for 2 weeks will be examined in a parallel experiment. Serial blood samples will be taken, and a blood level vs time curve will be plotted to compare the biological half-life of D+ in two groups. If the compound stimulates its own metabolism (e.g., polychlorinated or polybrominated biphenyls), a reduction in the biological half-life results. On the other hand, if the compound becomes stored in a third compound as a result of chronic exposure (e.g., tetracycline), its biological half-life will show an increase.

Metabolism

We will subject urine and fecal extracts to thin-layer chromatography using various solvent systems to obtain evidence for the metabolic

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conversion of D+. The samples will also be treated with β-glucuronidase and sulfatase before chromatography. Several organic solvents, singly or as mixtures, will be used to attempt to differentially extract D+ from its metabolites, if any. The thin-layer plate after solvent development will be autoradiographed or scanned on an autoscanner to locate radioactive spots. Extracts of tissues containing the highest level of radioactivity will be analyzed similarly.

Environmental Bioaccumulation and Magnification

This phase of the proposed investigation encompasses three tasks: Task 1 is to determine the extent to which D+ is assimilated into the tissues of selected freshwater organisms from water; Task 2 is to determine whether the concentration of D+ increases in biological tissues as it passes through an aquatic food chain; and Task 3 is to determine whether D+ is metabolized by aquatic organisms. These tasks will be performed in sequence, and the results from each task will determine the need to perform the next task in the sequence.

Task 1 - Direct Accumulation of D+ by Aquatic Organisms

All Task 1 experiments will be conducted under static conditions using saturated solutions of ^{14}C-labeled D+ containing about 600 dpm/ml of solution. We expect the concentration of D+ in a saturated aqueous solution to be in the part-per-billion range. Toxicity data provided by HRC indicate that the compound will not be acutely toxic at the proposed level.

For each organism studied, the tissues will be radioanalyzed at intervals during the exposure period to determine the rate of bioaccumulation, and bioaccumulation will be expressed in terms of the bioconcentration factor (BCF), which is the ratio of the concentration of the compound in the tissues and the concentration of the compound in the water when exposure is initiated.

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Algal Tests

We will use the single-celled green alga Selenastrum capricornutum, which we routinely culture in our laboratory. The alga will be exposed to D+ for 14 days in the usual culture medium and under the usual conditions of light and temperature. On days 2, 4, 8, and 14, the cultures will be sampled. The algal cells will be separated from the medium by vacuum filtration, washed, removed from the filter, weighed, and radioanalyzed. The test will be conducted in triplicate.

Invertebrate Tests

We recommend using the benthic worm Lumbriculus variegatus in place of the crustacean Daphnia magna in these tests because (1) we expect that in the aquatic environment, D+ will adsorb and concentrate in the sediments and thus accumulate more in benthic organisms than in the pelagic forms; and (2) if we proceed to Task 2, the worm will be more convenient to use because it is much larger than D. magna so the biomass needed to feed the fish would be easier to obtain. We would be pleased to use either organism, however. The cost of conducting Task 1 tests is the same for both organisms.

We will expose the worm or D. magna to D+ for 4 days. On days 1, 2, and 4, a sample of the population will be collected and the animals will be blot dried, weighed, and radioanalyzed. The test will be conducted in duplicate, and the animals will be exposed in 1 liter of D+ solution.

Fish Tests

The fish we plan to use is the bluegill sunfish (Lepomis machrochirus). The test also will be conducted in duplicate. Twelve fish will be exposed per duplicate to 19 liters of D+ solution for 4 days. On days 1, 2, and 4, four fish will be removed. Whole-body radioanalysis will be performed on two fish. With the other two, we will perform radioanalyses on excised muscle and visceral tissue.

Task 2 - Food Chain Biomagnification Study

We believe the Daphnia-bluegill biomagnification test described for pesticide registration (Federal Register 40, 26908, 1975) is more comprehensive than necessary for this study; however, we would be pleased to apply the test on request and at extra cost. The test is well described in the Register, so we will not describe it here except to note that it is a 32-day study on four groups of 10 fish (a total of 40 fish).

We propose to use a modified test in which fewer fish are used, and this will reduce the cost of preparing natural D+-contaminated food and the cost of radioanalysis.

We will feed 14 bluegills a daily ration of D+-contaminated Lumbriculus variegatus amounting to 10% of their average body weight for 28 days. The fish will be maintained in flowing water. The worms will be exposed to ^{14}C-labeled D+ under static conditions until their tissue residue is at least 80% of maximum, as estimated from the tests performed in Task 1. Before being fed to the fish, a sample of the exposed worms will be radioanalyzed to determine tissue residues. The daily ration will be provided all at once or several times a day, depending on the feeding rate. We will observe the feeding process to ensure that the entire ration is consumed.

On days 7, 14, 21, and 28, we will transfer three fish to clean flowing water and will prepare them for radioanalysis 48 hours later. Two fish will be left over at the end of the 28-day exposure period; these fish will be quick frozen and reserved for Task 3. After each sampling period, we will weigh the remaining fish in water and adjust the daily ration. A portion of the worms used in the study will be quick frozen and reserved for Task 3.

Task 3 - Metabolism of D+ by Aquatic Organisms

In Task 3, we will analyze the fish and worms reserved from Task 2 for D+ metabolites using the analytical procedures described for the mammalian metabolism study.

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Plant Uptake

Uptake from Water

We will determine the uptake of D+ from water by plants by growing soybean and tomato plants in a hydroponic system using a procedure based on one described in Growing Plants without Soil for Experimental Use (USDA Research Service, Misc. Publ. 1251, 1972). The seeds will be germinated on moist paper towels at 25° C in a dark room. After the seeds germinate, the roots of three plants will be inserted through a perforated jar lid, which when screwed to a jar allows the roots to hang in a nutrient solution containing ^{14}C-labeled D+.

We plan to use Hoagland and Arnon's nutrient solution containing about 1 uCi of ^{14}C-labeled D+ per liter. The solution will be gently aerated, and the plants will be grown for 3 weeks in an environmental chamber under controlled temperature, light, and humidity. At the end of 3 weeks, each plant will be weighed and radioanalyzed.

Uptake from Soil

If the soil percolation test shows that D+ adsorbs significantly to soil particles, we will determine the uptake of D+ from soil by soybean and tomato plants. The seeds of these plants will be germinated as described above. After germination, the seedlings will be transplanted to clay pots containing either sandy loam or a commercial soil mix and grown in the environmental chamber. Three times a week, we will water the plants with a solution of ^{14}C-labeled D+, containing 1 uCi/liter. At the end of 3 weeks, the plants will be harvested, washed, blot dried, weighed, and radioanalyzed.

Radioanalytical Methods

The procedures described here will be applied to all samples from all project phases in which ^{14}C-labeled D+ is used.

Aliquots (0.1 to 0.2 ml) of urine and plasma will be counted as such in a Searle Analytic Mark III liquid scintillation counter. A scintillation cocktail, such as Aquasol (New England Nuclear) or Scintisol

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(Isolab, Inc.) that can accommodate aqueous samples will be used. Colored samples will be decolorized with a few drops of 30% peroxide water. Fecal samples and whole organs will be weighed. Representative samples (1 to 2 g) will be taken from each organ and homogenized in three volumes of water. Aliquots of the homogenates will be dissolved in tissue solubilizers such as Unisol (Isolab, Inc.) or NCS (Amersham Searle) and then counted. Plants, algae, daphnia, and fish will be treated similarly to animal tissues for radioactivity assay.

Specimens in biodegradation and soil percolation studies will be subjected to differential solvent extractions using several nonpolar and polar organic solvents. Aliquots of these solvents will be counted after adding the scintillation cocktail.

Counting efficiencies in each type of sample will be assessed by the internal standard method.

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REPORTS

Within 30 days after completion of all experimental work, we will submit to HRC a report of our findings. That report will contain a description of the procedures we used, tabulations and discussions of the data collected, and recommendatioons for additional studies that we believe should be conducted.

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PROJECT ORGANIZATION AND PERSONNEL

Dr. David H. W. Liu, Manager of the Aquatic Toxicology Program, will be the Project Leader. He will be the primary SRI contact for HRC on the technical aspects of the study, and he will coordinate all the project research activities at SRI. Dr. Liu will also supervise the studies on accumulation and magnification of D+ by aquatic organisms.

Dr. Theodore Mill, Manager of the Physical Organic Chemistry Group, will supervise the work on D+ photolysis and oxidation. He will be assisted by Dr. William Mabey, Physical Organic Chemist.

Sediment/water partitioning and aqueous solubility studies will be performed under the direction of Dr. James H. Smith, Associate Manager, Analytical, Physical, and Inorganic Chemistry Group; and Dr. David C. Bomberger, Chemical Engineer, will direct the work on the degradation of D+ by sewage sludge.

The mammalian metabolism and residue studies will be performed under the direction of Dr. Chozo Mitoma, Director, Biomedical Research Department. He will also be in charge of all radioanalytical and thin-layer chromatographic activities.

Dr. Tsong-Wen Chou, an agricultural biologist, will conduct the plant uptake studies.

Biographies of all these participants on the project are appended.

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STATEMENT OF WORK

SRI International will provide the personnel, facilities, and equipment necessary to perform the work requested by Hooker Research Center in an appended letter dated 9 January 1978. Hooker Research Center will supply ^{14}C-labeled and nonlabeled Dechlorane Plus (D+) in the quantity and purity required. The quantity required will be discussed with HRC upon award of the contract; the required purity is discussed in the body of this proposal. Consistent with the request, all of the proposed experiments are designed to provide preliminary but essential information to determine whether D+ should be of environmental concern, and thus determine whether additional studies are necessary.

Environmental Fate Studies

These studies are designed to determine the potential transport and persistence of D+ in the physical environment. Experiments will be performed to determine its rate of photolysis and oxidation in aqueous systems and to determine whether the compound is altered upon exposure to sewage sludge. In addition, we will determine its aqueous solubility, its propensity to adsorb to sediment (sediment/water partitioning), and to soil (soil percolation test).

Environmental Effects Studies

Information provided by Hooker Research Center indicates that D+ shows very low acute toxicity in laboratory mammals and in aquatic organisms; therefore, the effects studies will be directed to determining its propensity to accumulate in the tissues of selected organisms.

Experiments will be performed to determine absorption, excretion, tissue distribution, and metabolism of D+ after oral administration in

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laboratory rats. We will also perform experiments to determine the extent to which selected aquatic organisms accumulate D+ directly from water and through an aquatic food chain and will determine whether D+ is metabolized by selected aquatic species. The uptake of D+ from water and soil by tomato and soybean plants also will be studied.

After completion of the laboratory studies, we will prepare for HRC a report of our findings. It will include a description of our experimental methods, a presentation and discussion of the data, and recommendations for additional studies.

CONTRACTUAL PROVISIONS

Proprietary Information

During the performance of the proposed study, certain information concerning the processes and business of the Hooker Research Center and affiliated organizations may become known to SRI project personnel. SRI will not divulge any proprietary information in accordance with the terms of its Confidential Information Agreement (SRI Form 1013) and its Standard Research Agreement (SRI Form 7001, paragraph 6). Three copies of each document are enclosed.

Estimated Time and Cost

Because the performance of some of the tests will depend on the data obtained from other tests, we cannot specify an exact period of performance. However, if we perform all the tests, the estimated period of performance is 4 months. The estimated charges for performing each phase of the project are as follows:

Mammalian Metabolism and Residue	$ 9,500
Degradation in the Environment	$11,500
Movement in Soil and Water	$20,000
Environmental Bioaccumulation/Magnification	$10,000
Plant Uptake	$ 2,250
Total	$53,250

In addition, a charge of $3,500 should be added for the cost of preparing the final report and for communications and miscellaneous expenses. The charges quoted would not be exceeded without written authorization from Hooker Research Center.

In view of SRI's status as a nonprofit organization, each research client is required to make an advance payment to cover working capital requirements for the project being undertaken. For a project of this

scope and duration, the advance payment is 50% of the aggregate charge for the work requested.

Project Authorization

This project can be authorized upon receipt of two signed copies of the Standard Research Agreement, two signed copies of the Confidential Information Agreement, and the advance payment.

Acceptance Period

This proposal will remain in effect until 15 April 1978; however, SRI would be pleased to consider an extension if requested.

Enclosures: Standard Research Agreement (3)
Confidential Information Agreement (3)

0 0 6 9

Appendix

BIOGRAPHIES

TSONG-WEN CHOU

Microbiologist-Biochemist
Pioneering Research
Life Sciences Division

SPECIALIZED PROFESSIONAL COMPETENCE

Biochemistry, microbiology, fermentations, food science and technology

REPRESENTATIVE RESEARCH ASSIGNMENTS AT SRI

Research in applied microbiology; daunorubicin fermentation; biodegradation of miscellaneous pollutants utilizing enrichment and cometabolic procedures; fermentation kinetics and biosorptions

OTHER PROFESSIONAL EXPERIENCE

Senior microbiologist, supervisor, Microbiology Laboratory, Rachelle Laboratories, Inc: strain improvement and optimization of tetracycline fermentation process at laboratory and pilot-plant scale; exploratory development work on bacitracin and lysine fermentations; biotransformation of tetracyclines

Research biochemist, University of California at Davis: study of metabolic pathway of ethylene biosynthesis by a mold

Research associate, Massachusetts Institute of Technology: single-cell protein research, especially on thermophilic hydrocarbon fermentation; microbial physiology and isolation characterization of metabolic products

Research assistant, Utah State University: physiological and biochemical effects on mold of gamma radiation

Research associate, Wei-Chuan Foods Company: research on fermentation production of glutamic acid; fermentative degradation of RNA to nucleotides; research on strain selection, optimum fermentation conditions and isolation purification of amino acids and nucleotides

ACADEMIC BACKGROUND

B.S. (1955) in agricultural chemistry, National Taiwan University; Ph.D. (1969) in food sciences and technology (food microbiology and biochemistry), Utah State University

PUBLICATIONS

Author of ten publications

PROFESSIONAL ASSOCIATIONS AND HONORS

American Chemical Society; American Society for Microbiologists; Institute of Food Technologists

Sigma Xi

August 1977

DAVID H. LIU

Manager, Aquatic Toxicology Program
Toxicology Department
Life Sciences Division

SPECIALIZED PROFESSIONAL COMPETENCE

Aquatic toxicology, invertebrates and fish; fish reproduction; pharmacology; biochemistry and metabolism of drugs

REPRESENTATIVE RESEARCH ASSIGNMENTS AT SRI (since 1972)

Determination of acute and chronic toxicity of pesticides to the bay mussel; study of benthic communities in San Francisco Bay; evaluation of published data on the toxicity of chemicals; development of procedures for studying the effects of pollutants on aquatic organisms; development of laboratory methods for the care and breeding of marine and freshwater organisms

OTHER PROFESSIONAL EXPERIENCE

Consultant, Las Virgines Water Sanitation District: design of continuous-flow bioassay systems

Senior aquatic biologist, Envirogenics Company: development of aquatic toxicology laboratory facilities, investigations on the acute and chronic toxicity of pesticides and other chemicals to freshwater fish and invertebrates

Aquatic biologist, General Electric Company/Battelle-Northwest Laboratories: chronic toxicity of ingested radionuclides in fish

USPHS postdoctoral fellow, Washington State University: metabolism in rodents, development of prophylactic and therapeutic methods for cyanide poisoning

USPHS trainee in toxicology, Oregon State University: radiorespirometric studies on pesticide effects on fish

ACADEMIC BACKGROUND

B.S. in zoology (1958), University of Michigan; M.A. in biology (1960), University of Oregon; Ph.D. in fisheries and toxicology (1969), Oregon State University

PUBLICATIONS

Author of eight technical publications

HONORS

Sigma Xi
USPHS Predoctoral Trainee in Toxicology (1965-69)
USPHS Postdoctoral Fellow in Pharmacology and Toxicology (1969-71)

April 1977

0 0 7 2

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Toxicology Department
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PUBLICATIONS

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HONORS

Sigma Xi
USPHS Predoctoral Trainee in Toxicology (1965-69)
USPHS Postdoctoral Fellow in Pharmacology and Toxicology (1969-71)

April 1977

0 0 7 3

CHOZO MITOMA

Director
Biomedical Research Department
Life Sciences Division

SPECIALIZED PROFESSIONAL COMPETENCE

Mechanism of action of chemical agents and psychotropic drugs; mechanism of and factors influencing drug metabolism; drug metabolite isolation and characterization

REPRESENTATIVE ADMINISTRATIVE AND RESEARCH ASSIGNMENTS AT SRI

Director, Biomedical Research Department (1969-present)
Senior biochemist
Biochemistry of the central nervous system, including the formation of 5-hydroxytryptophan from tryptophan, biosynthesis of λ-guanidinobutyric acid from λ-aminobutyric acid, and the effect of ortho- and meta-tyramines on the central nervous system and its implications in phenylketonuria
Mechanism of formation of collagen
Intermediary metabolism of amino acids
Detoxification studies of drugs using enzymologic and radioisotopic techniques
Biochemistry of the brain--effects of neurotoxic agents on the brain

OTHER PROFESSIONAL EXPERIENCE

Head, Section on Cellular Biochemistry, Laboratory of Clinical Biochemistry, National Heart Institute
Research biochemist, Laboratory of Chemical Pharmacology, National Heart Institute: research on hydroxylation reactions, hydroxyproline formation, drug metabolisms, and phenylketonuria
Rosalie B. Hite Fellow, Clayton Foundation Biochemical Institute, University of Texas: interconversion of purine bases and histidine
Research assistant, Biochemistry Department, University of California

ACADEMIC BACKGROUND

B.A. (1948) and Ph.D. (1951) in biochemistry, University of California (Berkeley)

PUBLICATIONS

Author or coauthor of over 80 technical publications

PROFESSIONAL ASSOCIATIONS AND HONORS

American Association for the Advancement of Science
American Society of Biological Chemists
American Society for Pharmacology and Experimental Therapeutics
Society for Experimental Biology and Medicine
Member of Editorial Board of Xenobiotica (since 1971)

April 1977

0074

SRI Proposal LSC 78-26(R)
3 March 1978

Dr. Arun K. Bhattacharya
Research and Development
HOOKER RESEARCH CENTER
Long Road
Grand Island, New York 14072

Dear Dr. Bhattacharya:

SRI International is pleased to submit this revision of SRI Proposal LSC 78-26 in response to the request of Dr. Paul Nees. The scope of the project has been reduced by deletion of the soil percolation studies and plant uptake experiments, and some of the protocols have been modified. Therefcre, the total estimated charge has also been reduced. The work we propose to perform is as follows:

- As originally proposed, we will determine the aqueous solubility of D+ because this information is essential for designing some of the other proposed experiments.

- We will determine the sediment-water partition coefficient as originally proposed.

- Oxidation and photolysis of D+ will be determined as originally proposed, but the rates will be determined from three measurements instead of five to eight.

- The study of mammalian metabolism and residues will be limited to the study of absorption. The absorption protocol will be the same as described in the original proposal, but we will use two doses and will analyze liver and kidney tissue for D+ 24 hours post administration. This approach will provide a minimum of information, but it will indicate whether a more comprehensive study on the uptake, distribution, retention, excretion, and metabolism of D+ by mammals is needed.

SRI International
333 Ravenswood Ave. • Menlo Park, California 94025
(415) 326-6200 • Cable: STANRES, Menlo Park • TWX: 910-373-1246

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· The environmental bioaccumulation/biomagnification studies will be limited to a screening study with fish. Several fish will be exposed to D+ for 96 hours. Half the population will be sacrificed, and the other half will be transferred to clean flowing water where they will be maintained for 96 hours and then sacrificed. Muscle tissue and viscera of both groups will be analyzed for D+. This approach will provide an estimate of the degree of D+ uptake and depuration by fish.

· The potential for biodegradation will be studied under aerobic and anaerobic conditions using activated sludge. We have increased the incubation to 6 weeks to allow time for the microorganisms to acclimate to D+. After 2 and 6 weeks, duplicate incubation flasks will be removed and the contents will be extracted with a mixture of hexane and isopropanol after disruption of the cells. The extract will be concentrated. An aliquot will be analyzed by liquid scintillation techniques, and another aliquot will be analyzed for the presence of metabolites using tlc and autoradiography. If at 2 weeks degradation is evident, the experiment will be terminated. Although the incubation time has been increased, the total effort involved is less than that of the originally proposed approach.

The contractual provisions presented in the original proposal will apply to this modification. The revised charges are as follows:

Mammalian Metabolism and Residue	$ 2,500
Degradation in the Environment	5,000
Movement in Soil and Water	11,200
Environmental Bioaccumulation/Magnification	1,600
TOTAL	$20,300

In addition, a charge of $1,700 should be added for the final report and for communications and miscellaneous expenses. The charges quoted would not be exceeded without written authorization from Hooker Research Center. The estimated performance period is 4 months, and the advance deposit required is the full amount.

Our revised price is somewhat higher than the maximum Dr. Nees informed me has been allocated for this investigation. Although he did not request changes in the sediment-water partitioning protocol, we believe that the protocol could be modified to reduce costs. We would be pleased to discuss these modifications with Dr. Nees when he attends the Society of Toxicology meeting in San Francisco on 13-16 March.

Respectfully submitted,

David H. W. Liu

David H. W. Liu, Ph.D., Manager
Aquatic Toxicology Program

Approved:

Gordon W. Newell, Ph.D., Director
Department of Toxicology

W. A. Skinner, Executive Director
Life Sciences Division

AGREEMENT

THIS AGREEMENT, entered into this ____ day of _____, 19___, by and between SRI International, a California nonprofit corporation, hereinafter referred to as SRI, and HOOKER RESEARCH CENTER

hereinafter referred to as the Research Client,

WITNESSETH:

WHEREAS the Research Client desires to have SRI perform the research services hereinafter set forth and SRI desires to perform such services,

NOW, THEREFORE, it is mutually agreed:

1. SRI shall conduct for the Research Client a research project relating to Metabolism and Environmental Screening Studies on ^{14}C-Labeled Dechlorane Plus (D+)

and more particularly set forth in SRI's Proposal No. LSC 78-26 dated 10 February 1978, which proposal by this reference is incorporated herein. SRI agrees to devote as much time and attention thereto as is reasonably required.

2. The Research Client will pay to SRI a sum not to exceed Fifty-six thousand seven hundred fifty dollars ($ 56,750), it being agreed that SRI will not incur expenses in excess of said amount, nor will the Research Client be obligated to reimburse SRI in excess of said amount unless a modification to this agreement shall have been entered into by the parties hereto.

3. Project charges reflect direct and indirect costs incurred in performance of the work, and an SRI fee.

4. Invoices shall be rendered to the Research Client by SRI as follows:

(a) The request for an advance deposit shall be presented upon execution of this agreement, and shall be for an amount previously determined as the working capital for this contract.

(b) Invoices for research services shall be rendered about every four weeks. The first such invoice shall cover the actual charges recorded since the beginning of the project, and succeeding invoices shall cover charges recorded since the effective date of the preceding invoice.

(c) When the accumulated total of invoices referred to in the above reaches the total authorized funds under this contract, less the initial deposit, subsequent invoices for research services shall be of a memorandum type. Each such invoice shall credit the amount of charges against the advance deposit.

(d) Upon completion of the project, a final invoice shall be rendered and any unexpended funds shall be returned to the Research Client. If and when SRI receives late charges of a significant amount from an external source after a final invoice has been submitted to the Research Client, SRI shall render a special invoice to the Research Client for the amount involved.

(e) All invoices submitted by SRI to the Research Client are due and payable upon receipt.

5. SRI agrees that all information obtained through work on the project shall be made available to the Research Client at any time, subject to the terms and conditions of this agreement, and that SRI will communicate promptly and without request all information which it deems pertinent to the project as it progresses.

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6. SRI represents that each of its employees has entered into a contract of employment which provides for assignment to SRI of all inventions made by the employee during the course of his employment.

SRI agrees that if during the period of this agreement any of its employees shall, to the knowledge of SRI, make a discovery or invention rising out of work on projects sponsored under this agreement, SRI will promptly make the fact of such discovery known to the Research Client. Upon the request of the Research Client, SRI agrees to use its best efforts to cause such employees to make application for letters patent. SRI further agrees to assign to the Research Client any and all rights SRI may have or may assert to such invention or discovery. The application for letters patent shall be made at the expense of the Research Client and through attorneys named by the Research Client and it is mutually agreed that any and all expenses, including staff time and travel for or in connection with the preparation, filing, prosecution, assignment and recording of such application, are payable by the Research Client. All such expenses incurred by SRI shall be separately invoiced to and paid by the Research Client without reference to the sums provided for in Paragraph 2 above.

7. The Research Client agrees that it will not use the name of SRI either expressed or implied in any of its advertising or sales promotional material. In the event Research Client intends to distribute outside of its own organization any report issued under this project, such report shall be used in its entirety, unless any proposed summary or abridgement of the report has been first approved by SRI. SRI agrees it will not publish or make known to others the results of said research investigation or information obtained therefrom without approval in writing from the Research Client. SRI agrees that unless specifically authorized in writing by the Research Client, it will not conduct for any other person during the term of this agreement investigations within the scope of the project.

8. SRI shall commence performance of this project on or about _____

and shall make every reasonable effort to complete such work by _____, unless the period of performance is extended by mutual agreement in writing. The Research Client agrees to give SRI written notice of its intentions regarding renewal of this agreement at least thirty (30) days prior to the estimated completion date.

9. The Research Client reserves the right to cancel this agreement but agrees to give thirty (30) days written notice to SRI of its election to so terminate. In such event, SRI will utilize its best effort immediately to curtail charges and commitments to this project.

IN WITNESS WHEREOF, the parties hereto have executed this agreement the day and year first above written.

SRI International

By _____

Title _____

_____

By _____

Title _____

SRI 7001 11/77

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SRI International	_____
By _____	By _____
Title _____	Title _____

SRI 7001 11/77

AGREEMENT

THIS AGREEMENT, effective _____, 19____, between STANFORD RESEARCH INSTITUTE (SRI) and _____ shall govern the conditions of disclosure by _____ of certain confidential information to be submitted to SRI relating to

With regard to the said information, SRI hereby agrees not knowingly to use such information, for itself or others, or to disclose such information to others (except to its employees who reasonably require same for the purpose hereof and who are bound to it by like obligation as to confidentiality) without the express written permission of _____ except that SRI shall not be prevented from using or disclosing information:

(a) previously known to SRI;
(b) subsequently otherwise legally acquired by SRI;
(c) in the public domain as of this date; or
(d) subsequently coming into the public domain.

Receipt of said information shall be without prejudice to SRI's right to contest the validity of any existing or future patent on the idea and shall not be deemed an admission of the novelty or patentability of the idea, or of priority or originality on the part of SRI or of the parties named in the said patent application.

THIS AGREEMENT shall remain in effect for a period of Three (3) Years from the date hereof.

STANFORD RESEARCH INSTITUTE,
a California corporation,

By _____

_____

By _____

SRI 1013
(Dec. 1968)

AGREEMENT

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SRI International

By _____

Title _____

_____

By _____

Title _____

SRI 7001 11/77

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STANFORD RESEARCH INSTITUTE,
a California corporation,

By _____

_____

By _____

SRI 1013
(Dec. 1968)

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STANFORD RESEARCH INSTITUTE,
a California corporation,

By _____

_____

By _____

SRI 1013
(Dec. 1968)

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